Effect of administration of an α_2 -adrenergic agonist, xylazine, on pulsatile gonadotrophin secretion in anoestrous horse mares

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Summary. Two experiments were performed to test the hypothesis that the seasonal suppression of gonadotrophin pulse frequency in anoestrous horse mares reflects inhibitory neural mechanisms. In a preliminary experiment (Exp. 1) conducted in February, 4 anoestrous mares were sedated by repeated intravenous injections of xylazine, an α_2 -adrenergic receptor agonist. On the day of treatment, 1–2 LH pulses were observed in xylazine-treated mares. In contrast, during a 12-h period only 1/8 untreated control mares exhibited a LH pulse. In Exp. 2, the effect of xylazine-induced sedation on pulsatile gonadotrophin release was examined in 4 anoestrous mares on two occasions before (18 November and 9 December) and after (23 December and 6 January) an abrupt, artificial increase in day length. Treatment with xylazine was associated with an overall increased FSH ($P < 0.01$) and LH ($P < 0.05$) pulse frequency, compared with that observed during 12-h pretreatment periods. To evaluate an effect of treatment at the various time during the experimental period, the change in FSH pulse frequency was analysed, since occasionally FSH pulses were unaccompanied by a change in serum LH values indicative of a LH pulse. On two occasions before increased daylength only 1/4 and 3/4 mares exhibited an increase in FSH pulses; in contrast, 14 days after increased daylength (23 December), 4/4 mares exhibited increased FSH pulse frequency associated with treatment. After 27 days of increased daylength (6 January), endogenous FSH pulse frequency was greater than before increased daylength and treatment with xylazine was unaccompanied by a further increase.

The results support the hypothesis that an inhibitory catecholaminergic neural system may play a role in the seasonal suppression of pulsatile gonadotrophin secretion in the anoestrous mare. The activity of this inhibitory system appears to decrease towards the approach of the breeding season.

Keywords: gonadotrophins; pulse frequency; seasonal inhibition; xylazine; anoestrous mares

Introduction

The breeding season of the horse mare begins in the spring, coinciding with an increase in daylength, and ends in the autumn as daylength decreases. A photoperiod-mediated increase of pulsatile luteinizing hormone (LH) pulse frequency has been implicated to play a role in the hormonal events leading to the initiation of the breeding season of horse mares (Fitzgerald *et al.*, 1987). The change in frequency of LH pulses during the transition from anoestrus to the breeding season occurs gradually, and the timing of the increase can be advanced by exposure of anoestrous mares to an abrupt, artificial increase in daylength. During anoestrus, when ovarian follicle development is minimal, LH pulse frequency is approximately once per 24 h and it is not uncommon for LH

pulses to be absent in secretory profiles of LH in individual anoestrous mares. Within 2 weeks before the first ovulation of the breeding season, LH pulse frequency increases to about 4–6 pulses per 24 h (Fitzgerald *et al.*, 1987). A further increase in LH pulse frequency probably occurs close to the time of ovulation, but this proposal has not been confirmed from changes in the pattern of secretion of LH in blood samples collected from the jugular vein (Fitzgerald *et al.*, 1987; Safir *et al.*, 1987). However, in blood samples collected via a cannula inserted into a facial vein, and with the tip positioned within the intercavernous sinus, and thus in close proximity to the pituitary gland, Alexander & Irvine (1987) reported that during the periovulatory period of the oestrous cycle gonadotrophin pulse frequency ranged between 0.45 and 1.87 pulses per h. In this experimental model, gonadotrophin pulses also were not readily detected in jugular vein blood. Thus for the mare, in some physiological or experimental conditions when frequent gonadotrophin pulses might be anticipated, collection of blood samples from the jugular vein may not provide evidence of pulsatile secretion (Alexander & Irvine, 1987; Fitzgerald *et al.*, 1983, 1985, 1987; Hines *et al.*, 1986, 1987; Safir *et al.*, 1987).

The mechanisms controlling the seasonal suppression of gonadotrophin pulse frequency in the anoestrous mare remain to be investigated. From recent studies conducted primarily on the sheep, a short-day breeder, a concept has been developed that seasonal anoestrus may reflect, in part, active neural inhibition of gonadotrophin-releasing hormone (GnRH) secretion from the hypothalamus. For example, in anoestrous ewes placement of lesions or knife cuts within the rostral region of the hypothalamus may lead a proportion of treated ewes to initiate oestrous cycles during the non-breeding season (Przekop & Domanski, 1980; Pau *et al.*, 1982; Pau & Jackson, 1985). These and related studies led Jackson *et al.* (1986) to propose that in a 'neutral state' the neuroendocrine mechanisms governing reproductive function in ewes are permanently 'switched on'. Additional support for this concept was provided by the studies of Meyer & Goodman (1985, 1986). In these studies, with drugs selected to antagonize or agonize potential neurotransmitters, an oestradiol-sensitive inhibitory catecholaminergic neural system was implicated to mediate the seasonal suppression of LH pulse frequency in intact anoestrous ewes.

The aim of the present study was to investigate the hypothesis that in anoestrous mares, as in the ewe, the seasonal suppression of LH pulse frequency may reflect an inhibitory neural mechanism. In these studies we used a pharmacological approach by examining the ability of xylazine administration to increase LH pulse frequency in the anoestrous mare. Xylazine, an α_2 -receptor agonist, was chosen since it is widely employed for the routine sedation of horses and, therefore, adverse side effects could be predicted.

Two related experiments were performed. The first study examined the ability of xylazine to increase LH pulse frequency in anoestrous mares. Based on this preliminary study, a second experiment tested the ability of xylazine to increase gonadotrophin pulse frequency in anoestrous mares before and after exposure to an abrupt, artificial increase in daylength. By manipulating photoperiod in this manner we attempted to increase endogenous gonadotrophin pulse frequency and thereby examine the hypothesis that xylazine would fail to increase further gonadotrophin pulse frequency, indicating decreased activity of the postulated inhibitory neural mechanism.

Materials and Methods

Experiment 1

Animals and treatment. Anoestrous mares, of mixed light horse breeds, were maintained on pasture (Lexington, KY 38°N) and given supplements with hay. Water was freely available. Additional hay and a small quantity of oats were given when the mares were housed in stalls for experimental procedures.

In February, 4 mares were sedated with xylazine for 6 h; the remaining 8 mares were untreated. Sedation was achieved by repeated injection (i.v.) of xylazine hydrochloride (100 mg/ml; Rompun, Haver, Miles Labs, Inc., Shawnee, Kansas, U.S.A.) in volumes which ranged between 2 and 6 ml per injection. The time and injection volume varied between individual animals to maintain a sedated condition characterized by a lowered head and evidence of

slight muscular relaxation. The mares remained standing throughout the experimental period. The final volume of xylazine administered to each mare ranged between 39 and 55 ml per 6 h.

Collection of blood samples. Blood samples (5 ml) were collected via a catheter inserted into a jugular vein, every 15 min for 1 h before, during and for 3 h after xylazine sedation or for 10 h in untreated mares. The catheter, which was also used for injection of xylazine, was inserted at least 1 h before blood collection and maintained patent by a removable stylet. Blood samples were allowed to clot at room temperature and stored overnight at 4°C. Serum was harvested and stored frozen until assayed for LH.

Experiment 2

Animals and treatments. Anoestrous mares of mixed light horse breeds were maintained on pasture and fed as described in Exp. 1. Beginning 9 December, the 4 mares were exposed to an abrupt, artificial increase in daylength. The mares were kept in stalls between 15:00 and 07:00 h each day and exposed to artificial lighting between 16:00 and 23:00 h, thereby extending daylength to at least 16 h light per day. The ability of xylazine, administered as described in Exp. 1, to increase gonadotrophin pulse frequency was examined on 2 occasions before (18 November and 9 November) and after (23 December and 6 January) the abrupt, artificial increase in daylength.

Collection of blood samples. On the day before treatment, blood samples (5 ml) were collected every 15 min for 12 h, beginning at 09:00 h. The next day xylazine was repeatedly injected for 6 h and blood samples were collected every 15 min during and for a further 6 h after stopping treatment. On 15 December a further 12-h period of frequent (every 15 min) blood collection was performed to obtain additional information regarding changes in endogenous gonadotrophin pulse frequency.

All blood samples were processed and stored as described in Exp. 1. In addition to LH, serum FSH concentrations were determined since we anticipated that, based on previous studies, pituitary LH content and response to GnRH in anoestrous mares would be lower than compared to the onset of the breeding season (Hart *et al.*, 1984; Silvia *et al.*, 1987), and so measurement of serum LH alone may not provide an accurate indication of changes in the pulsatile secretion of GnRH by the hypothalamus.

Radioimmunoassay of gonadotrophins

Serum concentrations of LH and FSH were determined by radioimmunoassay procedures as described previously (Fitzgerald *et al.*, 1983; Hines *et al.*, 1987). The sensitivity of the LH and FSH assays averaged 26 pg (LH, E98A) and 260 pg per tube (FSH, E99B). Interassay and intra-assay coefficients of variation were 14 and 19% and 13 and 8% for LH ($n = 12$) and FSH ($n = 8$) assays, respectively.

Statistical analysis

Gonadotrophin pulses were identified as described previously (Fitzgerald *et al.*, 1985). Briefly, the mean percentage coefficient of variation for LH or FSH values determined in the respective 10- or 12-h sampling period was calculated. When this value was at least 1.5-fold greater than the within-assay variation the gonadotrophin secretory profile was considered pulsatile. The mean percentage coefficient of variation of LH or FSH values comprising the ascending and descending portions of each potential LH or FSH pulse were similarly calculated. When this value was at least 1.5-fold greater than the within-assay variation, a pulse was identified. In each experiment, pulse frequency was calculated for each 10-h (Exp. 1) or 12-h (Exp. 2) period of frequent blood collection on the day of xylazine administration and compared with values obtained from appropriate non-injected controls. Gonadotrophin pulses observed during the 3-h (Exp. 1) or 4-h (Exp. 2) post-sedation periods were included in the analysis since xylazine is cleared relatively slowly from the circulation ($t_1 = 75$ min; Dyer *et al.*, 1987). Furthermore, in some mares behavioural signs of mild sedation were evident during the post-treatment periods. Data from Exp. 2 were subjected to a repeated measures analysis of variance (SAS, 1985). Orthogonal contrasts and protected LSD tests were used to compare means when a significant treatment effect was detected.

Results

In Exp. 1, xylazine-induced sedation of 3 mares was associated with 1–2 LH pulses and the pattern of secretion of LH for 2 mares is illustrated in Fig. 1. In the remaining mare an LH pulse was not observed during 5 h of administration of xylazine at which time patency of the cannula was lost. Upon collection of further blood samples 1 h later, serum LH values were elevated indicating that a LH pulse had occurred during the period that blood samples were not collected. Data from this mare were omitted in the comparison of LH pulse frequency between xylazine-treated and control animals. In this regard, in 7/8 untreated mares, serum LH values were low throughout the 10-h

period of frequent blood collection and LH pulses were absent. In the remaining mare 1 LH pulse was observed. Xylazine-induced sedation was, therefore associated with a greater mean LH pulse frequency compared to that observed in untreated mares (1.3 ± 0.3 versus 0.12 ± 0.12 (s.e.m.) pulses/10 h; $n = 3$ and 8, respectively).

Fig. 1. Changes in the pattern of secretion of LH in 2 mares from Exp. 1. Blood samples were collected at 15-min intervals and xylazine was repeatedly injected between Hours 1 and 7 of the 10-h collection period. The peak of an LH pulse is indicated (solid circle).

In Exp. 2, the ability of xylazine to increase gonadotrophin pulse frequency was examined in 4 anoestrous mares before and after exposure to an abrupt, artificial increase in daylength beginning 9 December. Overall, gonadotrophin pulse frequency was greater (LH, $P < 0.05$; FSH, $P < 0.01$) on the days of treatment with xylazine than during the 12-h pretreatment periods. Changes in the pattern of secretion of the gonadotrophins for 2 mares during each 12-h pretreatment period and on each day of xylazine sedation are depicted in Fig. 2. Occasionally, FSH pulses were unaccompanied by a change in serum LH values, indicative of an LH pulse according to the selected criteria (e.g. Mare 72, 18 Nov.). Therefore, to investigate changes in response to administration of xylazine before and after exposure to increased photoperiod, the pattern of secretion of FSH at the various times was evaluated.

Fig. 2. Changes in the pattern of secretion of LH and FSH for Mare 72 and Mare 58 from Exp. 2. Blood samples were collected at 15-min intervals for 12 h on the day before (17 Nov., 8 Dec., 22 Dec., 5 Jan.) and on the day of (18 Nov., 9 Dec., 23 Dec., 6 Jan.) treatment with xylazine. Xylazine was repeatedly injected between time 0 and 6 h. The peak of an LH or FSH pulse is indicated by the respective solid symbol. Beginning 9 December the mares were exposed to an abrupt, artificial increase in daylength (see 'Materials and Methods').

MARE 72

MARE 58

Between 17 November and 22 December (including 15 December) the pattern of secretion of FSH in all 4 mares during each pretreatment period was non-pulsatile. On 5 January, 27 days after exposure to increased daylength, all 4 mares exhibited 1–2 FSH pulses per 12 h (1.25 ± 0.25 versus 0 pulses/12 h; $P < 0.01$, 5 January and 17 November, respectively). Xylazine-induced sedation increased FSH pulse frequency, but not on all occasions during the experimental period. On 18 November, before exposure of the mares to an abrupt, artificial increase in daylength, 1 sedated mare exhibited 2 FSH pulses (Fig. 2) (0.5 ± 0.5 (s.e.m.) pulses per 12 h; $P > 0.05$) whereas in the remaining 3 mares the pattern of secretion was non-pulsatile. In 3/4 and 4/4 mares xylazine sedation was associated with 1–2 FSH pulses/12 h on 9 December (1.0 ± 0.4 (s.e.m.) pulses per 12 h; $P < 0.05$) and 23 December (1.5 ± 0.03 (s.e.m.) pulses per 12 h; $P < 0.05$), respectively. Finally, on 5 January at which time mean endogenous FSH pulse frequency was 1.25 pulses/12 h, xylazine-induced sedation was unaccompanied by a further increase in FSH pulse frequency (1.5 ± 0.3 (s.e.m.) pulses per 12 h; $P > 0.05$).

Discussion

Xylazine is classed as an α_2 -adrenergic receptor agonist, acting presynaptically to inhibit release of noradrenaline into the synaptic cleft of noradrenergic neurones (Freire & Leonardt, 1984; Hedler *et al.*, 1981). However, experiments which employ a pharmacological approach to the study of the mechanisms controlling hormone secretion must be interpreted with caution. A factor of concern is that no drug is entirely specific for a single class of receptors. Furthermore, primary effects of the selected drug on hormone secretion cannot be dissociated from those of secondary effects which may be a consequence of noradrenaline-induced alteration in another physiological process. A third factor of concern is the site of action of the selected drug. In this study, changes in the pattern of secretion of the gonadotrophins were interpreted as an index of changes in secretion of GnRH by the hypothalamus. Several studies on mares provide support for this concept (Garza *et al.*, 1986; Safir *et al.*, 1987), including comparison of changes in the pattern of GnRH and the gonadotrophins in blood samples collected from the intercavernous sinus (Alexander & Irvine, 1987). However, the possibility that xylazine may act directly on the anterior pituitary gland cannot be dismissed.

Notwithstanding the aforementioned considerations, the present results provide new information regarding the mechanisms controlling the seasonal suppression of pulsatile gonadotrophin secretion in anoestrous mares. The results of Exp. 1 suggest that in anoestrous mares xylazine-induced sedation leads to a transient increase in pulsatile LH secretion. This finding tentatively supports the hypothesis, proposed originally for the anoestrous ewe (Meyer & Goodman, 1985, 1986) that an inhibitory neural mechanism may be responsible for the seasonal suppression of pulsatile LH secretion. Since in this study we used xylazine, the inhibitory neural mechanism in horse mares may involve catecholaminergic neurones, in agreement with data from previous studies with the ewe.

In Exp. 1 we used anoestrous mares exposed to naturally increasing photoperiod. The low frequency of LH pulses observed in the untreated mares confirms previous observations from this laboratory (Fitzgerald *et al.*, 1987). In view of the significant increase in the frequency of pulsatile LH secretion induced by sedation with xylazine, the results obtained in Exp. 2 were unexpected: xylazine sedation of 4 mares on 2 occasions (18 November and 9 December) before exposure to an abrupt, artificial increase in daylength, was associated with the occurrence of pulsatile FSH secretion only in 1/4 and 3/4 mares, respectively. In contrast, on 23 December, 15 days after exposure to increased photoperiod, all mares exhibited a pulsatile pattern of FSH secretion on the day of xylazine-induced sedation. Interpretation of the apparent inability of xylazine to induce pulsatile FSH secretion in all mares before increased daylength is limited by the low number of animals used in this study. None the less, we might speculate that the apparent ability of xylazine to induce pulsatile FSH secretion in all mares after increased daylength, a result supported by

observations in Exp. 1, may reflect a change in the activity of the postulated inhibitory neural mechanism. Thus, before increased daylength the neural mechanism functions maximally and for some mares xylazine administration fails to block the effects of the inhibition. After exposure to increased daylength, this activity decreases and xylazine induces a further reduction leading to increased release of GnRH from the hypothalamus.

An alternative explanation might be that several inhibitory neural mechanisms act to suppress pulsatile GnRH secretion. For the ewe, a short-day breeder, two inhibitory neural mechanisms have been described. The steroid-dependent seasonal alteration in gonadotrophin secretion has been proposed to be mediated via catecholaminergic neurones, whereas the steroid-independent seasonal change in gonadotrophin secretion, evident in ovariectomized ewes, may involve an inhibitory serotonergic neural system (Meyer & Goodman, 1985, 1986). Conceivably, therefore, the apparent inability of xylazine to induce pulsatile gonadotrophin secretion in all mares before an increase in daylength may reflect a second additive neural mechanism which is not blocked by administration of xylazine.

It remains to be determined, therefore, whether one or more inhibitory mechanisms act to suppress pulsatile secretion of GnRH in the anoestrous mare. Furthermore, additional research is needed to determine whether the inhibitory catecholaminergic neural system, suggested by the results of the present studies, also functions in ovariectomized mares. In this regard, although results of previous studies have led to the hypothesis that the seasonal suppression of gonadotrophin secretion in mares occurs independently of a gonadal negative feedback action (Garcia *et al.*, 1979), the results obtained in this laboratory tentatively support an alternative hypothesis. In ovariectomized mares we observed a gradual increase in mean serum LH values during the spring months associated with increasing daylength (Fitzgerald *et al.*, 1983). For intact anoestrous mares, serum LH values remained low until a few days before the first ovulation of the breeding season (Fitzgerald *et al.*, 1987). Based on these indirect comparisons, it is possible that the ovaries exert a negative feedback action on gonadotrophin secretion in the anoestrous mare.

Finally, the apparent inability of xylazine to increase further the frequency of pulsatile FSH secretion in January, when endogenous FSH pulse frequency increased above values observed at the beginning of the study, supports the hypothesis that this change in endogenous gonadotrophin pulse frequency may reflect decreased activity of the postulated inhibitory neural mechanism. However, gonadotrophin pulse frequency in January was low, compared to gonadotrophin pulse frequencies previously reported for mares close to the start of the breeding season (Fitzgerald *et al.*, 1987; Hines *et al.*, 1987; Safir *et al.*, 1987). The neuroendocrine mechanisms responsible for further seasonal increases in gonadotrophin pulse frequency, particularly during the final weeks or days before ovulation, remain to be determined.

In conclusion, the results of this study extend earlier work on ewes and rams (Lincoln & Almeida, 1982) that an inhibitory neural system may play a role in the seasonal suppression of pulsatile GnRH secretion. In the anoestrous mare, the activity of this inhibitory neural system may vary during the non-breeding season, decreasing in activity towards the approach of the breeding season. It remains to be determined whether one or several inhibitory neural systems contribute to the seasonal suppression of pulsatile GnRH secretion in the anoestrous mare.

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